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Early postmortem electrical stimulation simulates PSE pork development☆

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Abstract

Carcasses from 64 gilts were subjected to electrical stimulation (ES) at 3, 15, 25, 35, 45, and 55 min postmortem or were untreated (NS). Temperature and pH of *longissimus* muscles were recorded at 1, 7, 14, 20, 30, 40, 50, and 60 min, and 24 h postmortem. Muscle samples were collected at 1, 30 and 60 min, and 24 h for determining glycolytic metabolite concentrations. ES at 3, 15, and 25 min resulted in lower ($P<0.05$) muscle pH, but stimulation after 25 min had no effect on muscle pH. Likewise, ES prior to 25 min resulted in greater ($P<0.05$) muscle temperatures. Muscle lactate concentrations were greater ($P<0.05$) in carcasses stimulated before 45 min postmortem. Glucose 6-phosphate concentration decreased ($P<0.05$) during the first hr postmortem and increased ($P<0.05$) thereafter. ES of carcasses at 45 and 55 min resulted in higher ($P<0.05$) concentrations of muscle glucose 6-phosphate at 24 h compared with NS and early-stimulated carcasses. Muscle glycogen concentrations at 30 min in carcasses stimulated at 3, 15 and 25 min were lower ($P<0.05$) than NS carcasses. Carcasses stimulated at 3 and 15 min exhibited lower ($P<0.05$) concentrations of muscle glycogen at 60 min than NS carcasses. Carcasses stimulated at 3 and 15 min postmortem exhibited lower ($P<0.05$) color and firmness scores, while ES at 3 and 25 min postmortem resulted in lower ($P<0.05$) water holding capacity. ES had no significant effect on CIE L^*, a^*, b^*, or 24 h muscle pH. These data show that ES of pork carcasses during the first 25 min postmortem creates PSE-like quality characteristics and suggest that ES is a potential model for studying pork quality development. © 2002 Published by Elsevier Science Ltd.

Keywords: Pork quality; Glycolysis; Electrical stimulation

1. Introduction

Pork quality and lack of product uniformity are major concerns of the pork industry worldwide; of particular concern is the development of pale, soft, exudative (PSE) pork or those products possessing these characteristics. Mechanisms controlling pork quality development are often associated with altered postmortem muscle metabolism. Specifically, changes in the extent or rate of glycolysis can create unfavorable pH and temperature combinations that result in muscle protein denaturation and diminished quality parameters (Bendall & Wismer-Pedersen, 1962; Briskey & Wismer-Pedersen, 1961; Wismer-Pedersen, 1959). Other than a limited number of genotypes, from a research standpoint the frequency of naturally occurring PSE is quite low (10–15%; Kauffman, Schrader, & Meeker, 1991), although in terms of industry this 10–15% represents a large number of carcasses and substantial economic loss. As a result, studies designed to elucidate the mechanisms controlling this phenomenon as they naturally occur are severely hampered by limited observations. Given that the industry is in the process of reducing the use of these genetics in commercial production facilities, yet still faces significant problems with PSE pork, methodologies are needed to develop PSE routinely in a controlled environment. Bowker, Wynveen, Grant, and Gerrard (1999) demonstrated that high levels (500 V) of electrical stimulation (ES) early postmortem (3 min) could be used to simulate PSE "-like" development in pork carcasses. ES was an effective insult that accelerated muscle pH decline early postmortem and generated PSE-like quality attributes. These researchers, however, did not address the time

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frame postmortem ES was most effective in triggering PSE-like development, and whether muscle metabolism postmortem resembled that commonly associated with PSE development. Therefore, the objective of this research was to determine when carcasses are most vulnerable to developing PSE induced via ES.

2. Materials and methods

2.1. Animals and design of experiment

Sixty-four pigs (average wt. 105 kg) from a single genetic source were obtained from the Pig Improvement Company (PIC, Franklin, KY, USA) and fed ad libitum prior to slaughter. Pigs were transported in small groups (~10/pen) to holding facilities approximately 16 h before slaughter where feed was withheld and animals were allowed water ad libitum. Pigs were slaughtered at the Purdue University Meat Science Research and Education Center. Exsanguination was considered zero min postmortem. Regardless of treatment, all carcasses were scalded at 5 min postmortem, followed by dehairing and evisceration.

Carcasses were randomly assigned to either electrical stimulation (ES) or nonstimulation (NS) treatments. Treated carcasses were subjected to ES (500 V, 26 pulses, 2 s on and 2 s off) at 3, 15, 25, 35, 45 or 55 min postmortem according to procedures established by Bowker et al. (1999). ES was administered through a 16.5 cm long steel electrode placed in the left shoulder muscles of the carcass with the rail serving as the ground. Carcasses were held at room temperature (~20 °C) until 60 min postmortem, at which time they were placed intact (unsplit) in a chill cooler (4 °C). To ensure that carcasses were handled similarly prior to the electrical stimulation treatment, all carcasses were split at 24 h postmortem.

2.2. Muscle pH and temperature measurements

Longissimus muscle pH values were recorded adjacent to the last rib at 1, 10, 14, 20, 30, 40, 50 and 60 min post-exsanguination using a Beckman Φ 110 ISFET pH meter with a spear-tipped KCl^- gel probe (Fullerton, CA, USA) that compensated for temperature differences. Meters were calibrated prior to and after measurement of every four carcasses using pH 4.00 and pH 7.00 buffers at 37 °C, and probes were cleaned by sequentially soaking in 10% bleach and 10% pepsin (0.1 g/ml; Fisher Scientific, Pittsburgh, PA, USA) solutions each for 10 min. The probes were inserted approximately 4.5 cm lateral to the midline of the carcass to a depth of approximately 5 cm at an angle perpendicular to the long axis of the *longissimus* muscle to ensure measurements were taken near the center of the muscle. Approximately 2 cm caudal from the site of pH measurement, temperature was measured using a VWR brand™ Traceable Digital Thermometer (Friendswood, TX, USA).

2.3. Meat quality characteristics

At 24 h postmortem, carcasses were split and ribbed between the 10th and 11th costae and color and firmness were evaluated (NPPC, 1991). Ultimate pH (pHu) was determined on the center of the cut surface of the *longissimus* muscle using a Beckman Φ 110 ISFET spear-tipped KCl^- gel probe. Two 2.54-cm thick chops were removed from the carcass at the 10th rib and trimmed to remove excess backfat and connective tissue. Objective color measurements were determined on one 2.54 cm chop using a Hunter Lab 45°/0° D25-PC2Δ Colorimeter (Hunter and Associates Laboratory Inc., Reston, VA, USA) with a 64 mm diameter port size. Mean CIE-L* (lightness), *a** (redness), *b** (yellowness) values were collected from three separate locations on the surface of each chop.

Water-holding capacity was determined on another chop using the drip loss method (Rasmussen & Stouffer, 1996). Briefly, muscle samples were collected from one of the 2.54-cm chops using a 2.54-cm diameter coring device. Samples were placed into the drip loss tubes so the cut surface of the meat was perpendicular to the long axis of the drip loss tube. Drip loss analysis was evaluated in triplicate from 7.0 g core samples. After 24 h at 4 °C the drip loss containers plus samples were reweighed. Muscle samples were removed and discarded and containers were re-weighed with exudate. Percentage drip loss was calculated and recorded.

2.4. Muscle sampling

Muscle samples were collected from the lumbar region of the *longissimus* muscle using a 1.3-cm diameter coring device at 1, 30, 60 min and 24 h postmortem. Samples were immediately frozen in liquid nitrogen and stored at −80 °C until analysis for muscle metabolite levels.

2.5. Muscle metabolite determination

Muscle glucose, glucose-6-phosphate, glycogen and lactate concentrations were determined using enzyme analytical methods (Bergmeyer, 1974; Dalrymple & Hamm, 1973) modified to a 96-well configuration. Frozen muscle samples (0.1–0.3 g) were ground using a mortar and pestle and homogenized using a Polytron (Brinkmann Instruments, Westbury, NY) in 10.0 ml of 0.5 M perchloric acid (PCA). A 500-μl extract aliquot was centrifuged (40,000 × ***g***) for 20 min. Supernatants from this extract were used for determination of

muscle glucose, glucose 6-phosphate and lactate concentrations.

An additional set of extracts (250 μl) was subjected to hydrolysis using 25 μl of KOH (30%) and 500 μl of amyloglucosidase (13.0 mg/ml; Sigma Chemical Company, St. Louis, MO) and incubated in a 37 °C water bath for three hours. Following incubation, 50 μl of 3.0 M PCA were added to stop hydrolysis. Samples were then centrifuged (40,000 × ***g***) for 15 min. Supernatants were recovered and used for muscle glycogen analysis.

For determination of muscle glucose and glucose 6-phosphate concentrations, 400 μl of extracted sample were added to 1700 μl 0.47 M triethanolamine (TEA) buffer in a 5-ml tube (borosilicate glass; Fisher Scientific, Hanover Park, IL). An aliquot (210 μl) was then added in triplicate to a 96-well plate (clear polystyrene; Fisher Scientific). Initial absorbance (OD_1) was recorded using a Spectracount™ microplate photometer (Packard Instrument Company, Downers Grove, IL, USA) with a 340-nm filter. Glucose 6-phosphate dehydrogenase (35 μl; 3.5 IU/sample; Fluka Chemical Company, St. Louis, MO, USA) was added to each tube and incubated for 5 min. Triplicate aliquots from each tube were transferred to 96-well plates and absorbance was measured again (OD_2) at 340 nm. For determination of glucose, hexokinase (20.4 μl; 2.04 IU/sample; Sigma Chemical) and ATP (40.7 μl; 11 mg/ml; Sigma Chemical) were added to each tube. Tubes were allowed to incubate for 5 min before triplicate aliquots were transferred to 96-well plates and absorbance (OD_3) measured at 340 nm. Absorbance values associated with muscle glucose 6-phosphate were calculated by subtracting OD_1 from OD_2. Similarly, absorbance values associated with muscle glucose were determined by subtracting OD_2 from OD_3. Quantities of muscle glucose and glucose 6-phosphate were determined from standard curves of 0, 50, 100 and 200 μM reduced nicotinamide adenine dinucleotide phosphate (NADPH; Sigma Chemical). Glucose and glucose 6-phosphate concentrations were reported as μmoles/g of muscle.

Muscle glycogen concentration was determined by adding 100 μl of hydrolyzed extract to 2000 μl of 0.1 M TEA buffer. Aliquots (210 μl) were transferred in triplicate to a 96-well plate and analyzed (OD_1). Glucose 6-phosphate dehydrogenase (35 μl), hexokinase (35 μl) and ATP (70 μl) were then added to the tube. Reactions were allowed to incubate for 10 min before triplicate aliquots from the tube were placed on a 96-well plate and absorbance (OD_2) measured at 340 nm. Absorbance values associated with muscle glycogen were determined by subtracting OD_1 from OD_2. Concentrations of muscle glycogen were determined from a standard curve of 0, 50, 100 and 150 μM NADPH. These values include glucose and glucose 6-phosphate intermediates that were not part of muscle glycogen analysis. Thus, glucose and glucose 6-phosphate concentrations from the previous assay were subtracted to determine only muscle glycogen. Glycogen concentration was reported as μmoles/g of muscle.

For determination of muscle lactate concentration, 100 μl of extract were added to 3000 μl of 0.1 M reaction buffer (200 mM *Tris*-hydrazine, 0.4 mM nicotinamide adenine dinucleotide, pH 9.0). Aliquots (210 μl) from each tube were transferred to 96-well plates (OD_1) and absorbance measured at 340 nm. Lactate dehydrogenase (40 μl; 4 IU/sample; Sigma Chemical) was then added to the buffered extract. Reactions were incubated for 2 h at 25°C before triplicate aliquots were placed on a 96-well plate and absorbance (OD_2) measured at 340 nm. Absorbance values associated with muscle lactate were determined by subtracting OD_1 from OD_2. Quantities of muscle lactate were determined from a standard curve of 0, 75, 150 and 300 μM NADH (Sigma Chemical). Lactate concentration was reported as μmoles/g of muscle.

Metabolite concentrations were used in the following equation to calculate glycolytic potential (μmoles/g of muscle; Monin & Sellier, 1985): 2(glucose + glucose-6-phosphate + glycogen) + lactate.

2.6. Data analysis

To evaluate the relationship between pH and temperature, glycolytic intermediates and meat quality characteristics, data were analyzed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (SAS, 1991). No differences were found between the initial pH and temperature of carcasses from different stimulation treatment groups (data not shown). Thus, data from NS carcasses were pooled until time of stimulation. Data were first analyzed for interactions among time of ES, pH and temperature to evaluate the effects of ES on muscle pH and temperature at different times postmortem. To evaluate the effects of ES at different times postmortem on muscle metabolites, data were analyzed using initial metabolite values as a covariate. In addition, interactions among initial metabolite levels within treatment, and initial metabolite levels within time of sampling were also tested.

3. Results

Muscle pH of carcasses stimulated at 3, 15 and 25 min postmortem was lower ($P < 0.05$) than NS carcasses from 20 to 60 min postmortem (Table 1). Carcasses stimulated after 25 min had muscle pH values similar to controls.

At various times postmortem, stimulated carcasses had elevated muscle temperatures (Table 2). Control and 3 min ES carcasses had similar muscle temperatures

Table 1
LS means (±SE) of *longissimus* muscle pH in stimulated and non-stimulated (NS) pork carcasses[a]

	Time postmortem (min)							
Trt	1	10	14	20	30	40	50	60
NS	6.77±0.01z ($n=64$)	6.58±0.01a, y ($n=56$)	6.44±0.01a, x ($n=56$)	6.36±0.01b, w ($n=45$)	6.27±0.01b, v ($n=35$)	6.17±0.02b, u ($n=24$)	6.03±0.04bc, t ($n=13$)	5.99±0.02de, t ($n=8$)
3 min ES		6.55±0.03a, z ($n=8$)	6.43±0.04a, y ($n=8$)	6.25±0.03a, x ($n=8$)	6.12±0.03a, w ($n=8$)	6.03±0.04a, w ($n=8$)	5.90±0.04a, v ($n=8$)	5.81±0.04ab, ($n=8$)
15 min ES				6.24±0.02a, z ($n=11$)	6.13±0.02a, y ($n=11$)	6.01±0.02a, x ($n=11$)	5.90±0.02a, w ($n=11$)	5.79±0.03a, v ($n=11$)
25 min ES					6.16±0.03a, z ($n=10$)	6.07±0.03a, y ($n=10$)	5.98±0.02ab, x ($n=10$)	5.89±0.02bc, w ($n=10$)
35 min ES						6.15±0.02b, z ($n=11$)	6.07±0.02cd, y ($n=11$)	5.96±0.02cde, x ($n=11$)
45 min ES							6.11±0.03d, z ($n=11$)	6.02±0.04e, y ($n=11$)
55 min ES								5.89±0.05bcd ($n=5$)

[a] LS means within a column with different letters (a, b, c, d) differ ($P<0.05$). LS means within a row with different letters (w, x, y, z) differ ($P<0.05$).

Table 2
LS means (±SE) of *longissimus* muscle temperature (C) in stimulated and non-stimulated (NS) pork carcasses[a]

	Time postmortem (min)							
Trt	1	10	14	20	30	40	50	60
NS	40.2±0.07xy ($n=64$)	40.3±0.08a, yz ($n=56$)	40.2±0.08a, xy ($n=56$)	40.4±0.09a, z ($n=45$)	40.2±0.09a, xyz ($n=35$)	40.0±0.14a, wx ($n=24$)	39.6±0.19a, vw ($n=13$)	39.3±0.29a, v ($n=8$)
3 min ES		40.4±0.14a, xy ($n=8$)	40.4±0.13a, xy ($n=8$)	40.9±0.04b, z ($n=8$)	40.8±0.06b, z ($n=8$)	40.6±0.07ab, yz ($n=8$)	40.2±0.11ab, x ($n=8$)	39.8±0.16ab, w ($n=8$)
15 min ES				40.6±0.21ab, z ($n=11$)	40.7±0.23b, z ($n=11$)	40.4±0.26ab, z ($n=11$)	40.1±0.28ab, yz ($n=11$)	39.7±0.31ab, y ($n=11$)
25 min ES					40.9±0.14b, z ($n=10$)	40.8±0.14b, z ($n=10$)	40.5±0.13b, yz ($n=10$)	40.1±0.15b, y ($n=10$)
35 min ES						40.0±0.14a, z ($n=11$)	39.7±0.20a, yz ($n=11$)	39.4±0.24a, y ($n=11$)
45 min ES							39.8±0.25a, z ($n=11$)	39.6±0.27ab, z ($n=11$)
55 min ES								39.1±0.32a ($n=5$)

[a] LS means within a column with different letters (a, b) differ ($P<0.05$). LS means within a row with different letters (v, w, x, y, z) differ ($P<0.05$).

during all times postmortem except at 20 and 30 min. Carcasses stimulated at 15 min had higher ($P<0.05$) temperatures at 30 min compared to NS carcasses. In addition, carcasses stimulated at 25 min had higher ($P<0.05$) muscle temperatures from 30 to 60 min postmortem than NS carcasses, but those stimulated after 25 min did not differ from NS carcasses.

Muscle glycogen concentrations in all carcasses decreased ($P<0.05$) with time postmortem (Table 3). Carcasses subjected to ES at 3, 15 and 25 min had lower ($P<0.05$) muscle glycogen concentrations at 30 min than NS carcasses. In addition, ES carcasses at 3 and 15 min had lower ($P<0.05$) levels of muscle glycogen at 60 min than NS carcasses, but did not differ from carcasses

Table 3
LS means (±pooled SE) of *longissimus* muscle glycogen concentrations (μmoles/g) in stimulated and non-stimulated (NS) pork carcasses[a]

	Time postmortem (min)			
Trt	1	30	60	1440
NS	68.77±2.36 z ($n=64$)	72.42±1.69 b,z ($n=35$)	51.58±3.58 cd,y ($n=8$)	12.54±3.58 a,x ($n=8$)
3 min ES		55.80±3.53 a,z ($n=8$)	34.70±3.53 a,y ($n=8$)	12.48±3.53 a,x ($n=8$)
15 min ES		56.71±3.05 a,z ($n=11$)	38.35±3.05 ab,y ($n=11$)	21.18±3.05 ab,x ($n=11$)
25 min ES		51.93±3.15 a,z ($n=10$)	45.00±3.01 bc,z ($n=10$)	23.54±3.01 b,y ($n=10$)
35 min ES			47.30±3.02 c,z ($n=11$)	22.87±3.02 b,y ($n=11$)
45 min ES			65.75±3.01 e,z ($n=11$)	21.37±3.01 ab,y ($n=11$)
55 min ES			59.58±4.64 de,z ($n=5$)	11.33±4.64 a,y ($n=5$)

[a] LS means within a column with different letters (a,b,c,d,e) differ ($P<0.05$). LS means within a row with different letters (x,y,z) differ ($P<0.05$).

Table 4
LS means (±pooled SE) of *longissimus* muscle glucose concentrations (μmoles/g) in stimulated and non-stimulated (NS) pork carcasses[a]

Time postmortem (min)				
Trt	1	30	60	1440
NS	5.07±0.21 y ($n=64$)	5.33±0.20 a,y ($n=35$)	6.22±0.42 ab,y ($n=8$)	10.36±0.42 a,z ($n=8$)
3 min ES		5.86±0.43 ab,x ($n=8$)	9.60±0.43 e,y ($n=8$)	11.71±0.43 b,z ($n=8$)
15 min ES		7.49±0.36 c,y ($n=11$)	8.40±0.36 d,y ($n=11$)	11.03±0.36 ab,z ($n=11$)
25 min ES		6.34±0.38 b,x ($n=10$)	7.94±0.36 cd,y ($n=10$)	10.50±0.36 a,z ($n=10$)
35 min ES			7.31±0.38 bc,y ($n=11$)	10.46±0.38 a,z ($n=11$)
45 min ES			6.05±0.37 a,y ($n=11$)	11.26±0.37 ab,z ($n=11$)
55 min ES			7.02±0.70 abcd,y ($n=5$)	11.19±0.70 ab,z ($n=5$)

[a] LS means within a column with different letters (a,b,c,d) differ ($P<0.05$). LS means within a row with different letters (x,y,z) differ ($P<0.05$).

electrically stimulated at 25 and 35 min. In contrast, electrical stimulation at 45 min resulted in higher ($P<0.05$) concentrations of muscle glycogen measured at 60 min than stimulation at 3, 15, 25, 35 min or no stimulation, but did not differ from stimulation at 55 min. Stimulation of carcasses at 25 and 35 min resulted in higher ($P<0.05$) concentrations of muscle glycogen at 24 h than stimulation at 3, 15, 55 min and NS treatments, but was not different from 45 min stimulation carcasses.

Muscle glucose concentrations increased ($P<0.001$) with time postmortem across all treatments (Table 4). Muscle glucose concentration was higher ($P<0.05$) in carcasses stimulated at 3 and 15 min postmortem than controls at 30 min. Carcasses subjected to ES between 3 and 25 min had higher ($P<0.05$) concentrations of glucose in the muscle at 60 min. Stimulation of carcasses at 3 min resulted in higher ($P<0.05$) muscle glucose concentrations at 60 min and 24 h than NS carcasses.

With the exception of carcasses stimulated at 3 min, muscle glucose 6-phosphate (G6P) concentration in all carcasses decreased (Table 5) during the first hour postmortem. Between 60 min and 24 h, muscle G6P concentration increased ($P<0.05$) in NS carcasses and

Table 5
LS means (±pooled SE) of *longissimus* muscle glucose 6-phosphate concentrations (μmoles/g) in stimulated and non-stimulated (NS) pork carcasses[a]

	Time postmortem (min)			
Trt	1	30	60	1440
NS	14.18±0.60 z ($n=64$)	12.85±0.51 a,yz ($n=35$)	8.24±1.04 ab,x ($n=8$)	11.25±1.04 a,y ($n=8$)
3 min ES		12.33±1.10 a,z ($n=8$)	10.00±1.10 ab,z ($n=8$)	11.64±1.10 ab,z ($n=8$)
15 min ES		12.37±0.92 a,z ($n=11$)	9.60±0.92 ab,y ($n=11$)	11.93±0.92 ab,yz ($n=11$)
25 min ES		12.31±0.93 a,z ($n=10$)	9.32±0.90 ab,y ($n=10$)	10.74±0.90 a,yz ($n=10$)
35 min ES			10.76±0.92 b,y ($n=11$)	11.84±0.92 ab,z ($n=11$)
45 min ES			7.85±0.90 a,y ($n=11$)	14.21±0.90 bc,z ($n=11$)
55 min ES			9.82±1.33 ab,y ($n=5$)	15.73±1.33 c,z ($n=5$)

[a] LS means within a column with different letters (a,b,c) differ ($P<0.05$). LS means within a row with different letters (x,y,z) differ ($P<0.05$).

Table 6
LS means (±pooled SE) of *longissimus* muscle lactate concentrations (μmoles/g) in stimulated and non-stimulated (NS) pork carcasses[a]

	Time postmortem (min)			
Trt	1	30	60	1440
NS	43.85±1.99 w ($n=64$)	52.74±1.84 a,x ($n=35$)	72.97±4.03 ab,y ($n=8$)	109.47±4.03 a,z ($n=8$)
3 min ES		62.12±3.80 b,x ($n=8$)	103.47±3.80 c,y ($n=8$)	119.14±3.80 a,z ($n=8$)
15 min ES		73.90±3.31 c,x ($n=11$)	97.47±3.32 c,y ($n=11$)	113.05±3.32 a,z ($n=11$)
25 min ES		65.59±3.40 c,x ($n=10$)	82.80±3.25 b,y ($n=10$)	113.55±3.25 a,z ($n=10$)
35 min ES			75.45±3.26 b,y ($n=11$)	113.46±3.26 a,z ($n=11$)
45 min ES			65.82±3.27 a,y ($n=11$)	112.23±3.27 a,z ($n=11$)
55 min ES			71.26±4.84 a,y ($n=5$)	107.19±4.84 a,z ($n=5$)

[a] LS means within a column with different letters (a,b,c) differ ($P<0.05$). LS means within a row with different letters (w,x,y,z) differ ($P<0.05$).

those stimulated after 25 min. Carcasses stimulated after 35 min had greater ($P<0.05$) concentrations of G6P than controls at 24 h.

Across all treatments, muscle lactate concentrations increased ($P<0.001$) with time postmortem (Table 6). Muscle lactate concentrations were higher ($P<0.05$) at 30 min in carcasses stimulated at 3, 15 and 25 min postmortem, but only 3 and 15 min stimulated carcasses had higher ($P<0.05$) lactate concentrations at 60 min. Muscle lactate concentrations at 24 h were not different across any treatment combination.

For the most part, calculated muscle GP values decreased with time postmortem (Table 7). Muscle samples taken at 60 min and 24 h from NS carcasses did not differ in GP. However, muscle GP values at 1, 60 min and 24 h were lower ($P<0.05$) than 30 min. Muscle GP values of 3 and 25 min ES carcasses had similar GP values across all sampling times. ES at 45 and 55 min

had lower ($P<0.05$) GP values at 60 min and 24 h. ES at 3 and 25 min resulted in lower ($P<0.05$) GP values than NS at 30 min postmortem, but stimulation at 3 and 15 min had similar GP values. Muscle samples of carcasses stimulated at 45 min had higher ($P<0.05$) GP values than those stimulated at 25, 35 min and NS carcasses, but were not different from carcasses stimulated at 3, 15 and 55 min. Carcasses subjected to ES at 15, 25, 35 and 45 min had higher ($P<0.05$) GP values than NS and 55 min treatments.

Only carcasses stimulated at 3 and 15 min had lower ($P<0.05$) color scores than NS carcasses (Table 8). Similarly, only carcasses stimulated at 3 and 15 min had reduced ($P<0.05$) firmness scores compared with controls. ES and NS carcasses did not differ with respect to CIE L^*, a^*, b^* or ultimate muscle pH. However, carcasses stimulated at 3 and 25 min postmortem had higher ($P<0.05$) drip loss than NS carcasses.

4. Discussion

Adverse pork quality development is driven by a pH and temperature related phenomenon occurring early postmortem. As a result, any perturbation that directly or indirectly alters one or both of these parameters during the transformation of muscle to meat can exacerbate adverse pork quality. Several researchers have shown that subjecting pork carcasses to ES accelerates pH decline and creates pale pork (Bowker et al., 1999; Forrest & Briskey, 1967; Forrest, Judge, Sink, Hoekstra & Briskey, 1966), yet others have shown ES has little effect on meat quality parameters (Gigiel & James, 1984; Johnson, Savell, Weatherspoon & Smith, 1982; Westervelt & Stouffer, 1978). These discrepancies in the literature likely result from differences in methodologies, namely time of stimulation postmortem or potentially, genetic background. Utilizing these

Table 7
LS means (±pooled SE) of *longissimus* muscle glycolytic potential values in stimulated and non-stimulated (NS) pork carcasses[a]

Time postmortem (min) Trt	1	30	60	1440
NS	219.88±5.67 y ($n=64$)	235.60±3.55 c,z ($n=35$)	203.60±7.76 a,x ($n=8$)	182.58±7.76 a,x ($n=8$)
3 min ES		207.29±7.42 ab,z ($n=8$)	210.26±6.35 ab,z ($n=8$)	190.93±7.42 ab,z ($n=8$)
15 min ES		225.26±6.35 bc,z ($n=11$)	210.85±6.35 ab,yz ($n=11$)	203.56±6.35 b,y ($n=11$)
25 min ES		206.24±6.64 a,z ($n=10$)	207.84±6.35 a,z ($n=10$)	203.39±6.35 b,z ($n=10$)
35 min ES			207.62±6.35 a,z ($n=11$)	202.69±6.35 b,z ($n=11$)
45 min ES			226.54±6.36 b,z ($n=11$)	205.15±6.36 b,y ($n=11$)
55 min ES			222.85±9.74 ab,z ($n=5$)	176.05±9.74 a,y ($n=5$)

[a] LS means within a column with different letters (a,b,c) differ ($P<0.05$). LS means within a row with different letters (x,y,z) differ ($P<0.05$).

Table 8
LS means (±SE) of *longissimus* muscle meat quality characteristics in stimulated and non-stimulated (NS) pork carcasses[a]

	Treatments						
Quality parameter	NS	3 min ES	15 min ES	25 min ES	35 min ES	45 min ES	55 min ES
Color	2.69±0.13b	2.13±0.18a	2.30±0.13a	2.36±0.12ab	2.59±0.11ab	2.68±0.10b	2.50±0.16ab
Firmness	2.88±0.23c	1.88±0.28a	2.30±0.17b	2.41±0.16bc	2.45±0.16bc	2.64±0.12bc	2.60±0.10bc
L^*	60.0±0.51abc	61.9±1.06bc	62.1±0.82c	61.6±0.85bc	60.0±0.69ab	59.2±0.59a	59.5±0.87ab
a^*	5.7±0.74ab	4.7±0.77a	5.6±0.72a	6.1±0.42ab	6.0±0.49ab	7.2±0.42b	6.6±0.51ab
b^*	14.5±0.23ab	15.1±0.45ab	15.3±0.35b	14.6±0.27ab	14.7±0.22ab	14.3±0.32a	14.1±0.31a
pHu	5.60±0.03a	5.61±0.02a	5.57±0.02a	5.61±0.02a	5.59±0.02a	5.60±0.01a	5.63±0.04a
Drip loss (%)	3.66±0.51a	6.15±0.72b	4.93±0.36ab	5.74±0.63b	4.69±0.56ab	4.84±0.48ab	4.64±0.75ab

[a] LS means within a row with different letters (a,b,c) differ ($P<0.05$). Color score of 1 = pale pinkish gray to white, 5 = dark purplish red; firmness score of 1 = very soft, 5 = very firm; L^* = Lightness; a^* = redness; b^* = yellowness.

conflicting data, we hypothesized that muscle may be more susceptible or refractile to PSE development at different times postmortem. To test this hypothesis, we challenged postmortem muscle metabolism of pork carcasses with ES in an attempt to define the time when pork carcasses are most vulnerable to PSE development. Data presented here show that application of ES to pork carcasses at or before 25 min adversely alters the normal evolution of muscle pH and temperature and enhances postmortem muscle glycolysis. Although not in absolute agreement, objective and subjective pork quality scores run congruent to these changes. Furthermore, these data show that there is a fixed period during which pork carcasses are vulnerable to PSE development, and suggest that ES is a valuable model for studying this aberrant pork quality problem. Whether these times are consistent across genetic populations is unknown but may be of interest to those charged with developing genetic populations that yield high quality pork.

Validating electrical stimulation as a model to study "classical" PSE development, either arising from genetic populations inherently "susceptible" to adverse pork quality or that naturally occur in the absence of major gene influences, remains a point of serious concern. Given the metabolic profiles provided from this study and those provided by Kastenschmidt, Hoekstra, and Briskey (1968), it appears that adverse pork quality developed using ES closely mimics metabolism exhibited by the "slow-glycolyzing" rather than the "fast-glycolyzing" muscle. The fast-glycolyzing muscle used in these studies, in retrospect, was thought to be a result of the halothane (HAL) gene, even though definitive tests for presence of the HAL or rendement napole (RN) genes were not performed on their pigs. Therefore, reduced pork quality via ES likely develops differently than that occurring in HAL pigs.

Biochemical studies of this nature are crucial for determining which enzyme in postmortem muscle is rate limiting, implying that only one enzyme may be responsible for aggressive glycolysis. Although this rationale reflects theoretical biochemical kinetics, our data suggest that more than one glycolytic enzyme can be 'rate-limiting' during postmortem muscle metabolism. Certainly, it is impossible for multiple enzymes to be rate limiting simultaneously, but data suggest that different enzymes may be rate limiting at different times postmortem. Our data shows that G6P decreases during the first hour postmortem, then increases afterwards. If sampling methods were restricted to a single time after exsanguination, interpretation of our data would have been different depending on whether samples were taken prior to or after an hour postmortem. These data show the importance of intensive sampling for understanding fully changes in postmortem muscle glycolysis and suggest more than one enzyme may be rate-limiting during the transformation of muscle to meat.

As mentioned earlier, our data is similar to that reported by Kastenschmidt et al. (1968), who observed G6P decline during the first hour postmortem and rebound to levels concentrations equal to or higher than the earliest point sampled. This decrease in G6P was not, however, observed in the "fast glycolyzing" Poland China pigs that were likely HAL positive. The finding that G6P decreased in muscle from ES carcasses suggests that glycogen phosphorylase is rate limiting during the first hour postmortem in the ES model. Glycogen phosphorylase, a glycogenolytic enzyme responsible for cleaving glucose residues from glycogen, may be partially responsible for the accumulation of G6P after 1 h postmortem and is a key regulator of glycolysis (Dalrymple & Hamm, 1975; Kastenschmidt et al., 1968; Scopes, 1973). Under conditions used in this study, it is possible that glycogen phosphorylase was incapable of supplying sufficient G6P for distal reactions of the glycolytic pathway, and therefore the pool of G6P is rapidly metabolized. The accumulation observed after 60 min may have been a result of increased glycogen breakdown (via glycogen phosphorylase), or a decrease in the activity of phosphofructokinase (PFK) or another rate-limiting enzyme in glycolysis (Scopes, 1974). Intuitively, the latter possibility seems most logical given glycolysis obviously slows with time; however, the observation that muscle of carcasses stimulated at 45 and 55 min postmortem accumulates greater quantities of G6P by 24 h postmortem suggests that glycogen phosphorylase may be responding to ES.

In early postmortem muscle, glycogen phosphorylase exists in an active and inactive form as glycogen phosphorylase a and b, respectively (Scopes, 1974). Glycogen phosphorylase b is inhibited by high levels of ATP and G6P, whereas glycogen phosphorylase a is not. Furthermore, in a state of homeostasis, the major form of glycogen phosphorylase exists in the inactive form in muscle. Therefore, assuming that antemortem handling is not overly harsh, the relative abundance of active glycogen phosphorylase would be rather low. After an animal is stunned, the muscle would attempt to maintain homeostatic control of energy metabolism for a period of time, after which ATP and G6P levels would fall and AMP concentrations would increase. As a result, the allosteric effects of ATP and G6P on phosphorylase b would be lost and phosphorylase b would be active. This loss in enzyme inhibition may be partially responsible for increased accumulation of G6P in the muscle after 1 h. Additional work will be necessary to test this possibility.

Our data supports PFK as a rate-limiting enzyme in glycolysis, at least in the latter phases of postmortem metabolism. Conversion of fructose 6-phosphate to fructose 1,6-bisphsophate is a committed step in glycolysis and is catalyzed by PFK. ATP is needed by PFK to initiate the transfer of a phosphate group from ATP to

fructose 6-phosphate. Kastenschmidt et al. (1968) demonstrated that ATP levels decrease dramatically postmortem, especially in "fast-glycolyzing" muscle. This suggests that the ability of PFK to catalyze the formation of 1, 6-bisphosphate may be hindered from lowered amounts of muscle ATP later postmortem. Unfortunately, we did not generate data between 60 min and 24 h postmortem, or evaluate additional metabolites (downstream of G6P). Additional work will be necessary for determining the exact time when G6P begins to accumulate in postmortem muscle and whether pyruvate kinase, another rate-limiting enzyme, may be controlling glycolysis in a time-dependant manner during the transformation of muscle to meat. Expanding sampling to include these times will likely delineate the rate-limiting steps of glycogenolysis and glycolysis that may be modulating development of PSE pork in ES carcasses. Taken together, data presented here show that ES is an effective insult for triggering abnormal muscle pH and temperature declines that are associated with altered muscle metabolism and lean tissue that is lighter colored, less firm and more exudative. The exact mechanisms underlying these abnormalities in the transformation of muscle to meat are not known but are likely different than those shown for halothane positive, fast-glycolyzing muscle. Understanding these mechanisms should prove useful in the quest for unraveling the complex machinery controlling pork quality development.

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